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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Heregulin (HRG) is a growth factor that activates *erbB-2-3-4* receptors. HRG is expressed in about 30% of breast cancer tumors where it is inversely correlated with expression of the estrogen receptor (ER). Tumors that express ER are less aggressive than non-expressing tumors and they respond to anti-estrogens. Interestingly, HRG induces loss of ER function. We have generated a novel model of tumor progression from a hormone-dependent to a hormone-independent phenotype by introducing HRG into breast cancer cells. The resulting cells were anti-estrogen resistant and metastatic. We now would like to investigate the mechanism by which HRG induces tumor progression. **Our working hypothesis is that expression of HRG induces an uncontrolled mitogen activated protein kinase (MAPK) cascade producing unbalanced growth promoting genes. This uncontrolled cascade results in the development of an aggressive phenotype and resistance to Tamoxifen (Tam). The specific aims of this proposal are to determine the relationship between HRG induction of MAPK activity and the resulting induction of Tam resistance and to restore hormonal-response. Our studies will provide information on how the MAPK cascade influences the outcome of HRG's biological action. Interventions to block this cascade and restore hormonal action will demonstrate the mechanism by which HRG induces an hormone-independent phenotype. Ultimately, elucidation of the ability of pharmacological agents to revert the aggressive phenotype induced by HRG will be beneficial for a large population of breast cancer patients who are resistant to anti-estrogen therapies.**

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INTRODUCTION

In the last decade, numerous studies have indicated that polypeptide growth factors and their receptors play an important role in breast cancer and have mitogenic effects. One such family constitutes the heregulins (HRGs), EGF-like growth factors that bind directly to the erbB3 and erbB4 receptors and induce tyrosine phosphorylation of erbB2 via receptor heterodimerization. Previous studies from our laboratory, have demonstrated that HRG-β2 induces estrogen-independent growth, estrogen receptor down-regulation, enhances cell proliferation, tumorigenicity and metastasis when stably transfected into estrogen receptor positive MCF-7 breast cancer cells. Some evidence suggests a role for the Ras-Raf-MAPK pathway in HRG signaling. HRG induces tyrosine phosphorylation of SHC and its association with GRB2-SOS complex, which in turn can activate $p21^{ras}$. It also activates a down stream target of ras, p42/44 MAPK. The Ras-Raf-MAPK pathway is required for proliferative response to many growth factors and hormones. A cross talk has also been established between MAPK and estrogen receptor (ER), since MAPK can activate ER independent of estrogen by stimulating its phosphorylation at Ser 118. In this study, we have used a GDP-bound dominant negative mutant of ras (N17) in order to determine the involvement of Ras/-Raf-MAPK pathway in acquisition of HRG-induced estrogen-independent phenotype.

BODY:

Heregulin (HRG) is a growth factor that activates *erbB-2-3-4* receptors. HRG is expressed in about 30% of breast cancer tumors where it is inversely correlated with expression of the estrogen receptor (ER). Tumors that express ER are less aggressive than non-expressing tumors and they respond to anti-estrogens. Interestingly, HRG induces loss of ER function. We have generated a novel model of tumor progression from a hormone-dependent to a hormone-independent phenotype by introducing HRG into breast cancer cells. The resulting cells were anti-estrogen resistant and metastatic. We now would like to investigate the mechanism by which HRG induces tumor progression.

Clinical studies have shown that *erbB-2* oncogene product, when overexpressed, correlates with tamoxifen resistance in ER positive breast cancer specimens. The response rate to tamoxifen in the metastatic setting varies from 50-75% in ER positive patients. In patients whose tumors overexpress *erbB-2* in the context of the ER receptor, this response rate decreases to 17%. Although the presence of estrogen receptor is employed to predict the hormone dependency of a tumor, the relationship with response to endocrine therapy is not absolute. Significant levels of estrogen receptor have been detected in more than 60% of human breast cancers, at best only two-thirds of these ER positive tumors respond to endocrine therapy. Why this should occur is unclear, however our experimental studies have demonstrated a relationship between the ER and *erbB-2* signaling pathways. For example, it has also been shown that estradiol will down

regulate *erbB-2* in overexpressing cells and that ER is required for this to occur. Potentiation of breast cancer cell growth by either the ER or the *erbB*-pathway may make cells less amenable to anti-proliferative strategies directed to the alternative pathway.

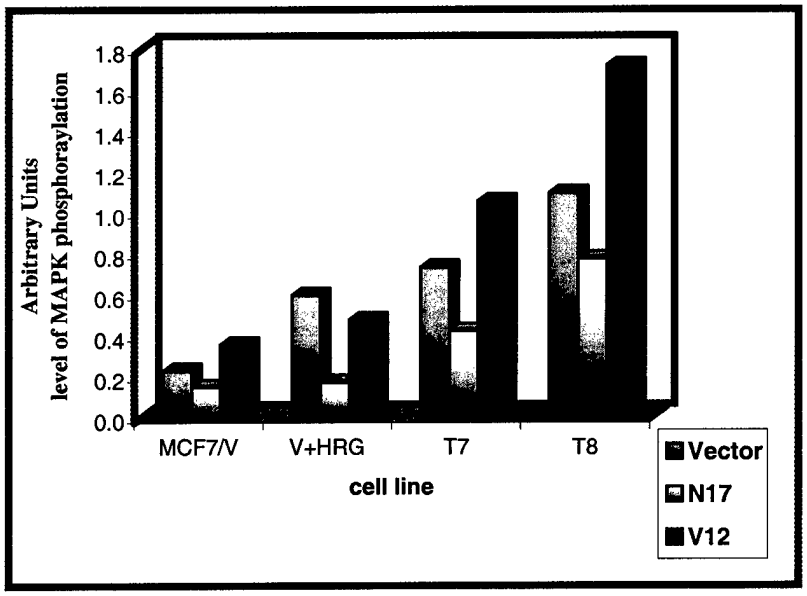
Our working hypothesis was that expression of HRG induces an uncontrolled mitogen activated protein kinase (MAPK) cascade producing unbalanced growth promoting genes. This uncontrolled cascade results in the development of an aggressive phenotype and resistance to Tamoxifen (Tam). The specific aims of this proposal are to determine the relationship between HRG induction of MAPK activity and the resulting induction of Tam resistance and to restore hormonal-response.

Our studies will provide information on how the MAPK cascade influences the outcome of HRG's biological action. Interventions to block this cascade and restore hormonal action will demonstrate the mechanism by which HRG induces a hormone-independent phenotype. Ultimately, elucidation of the ability of pharmacological agents to revert the aggressive phenotype induced by HRG will be beneficial for a large population of breast cancer patients who are resistant to anti-estrogen therapies.

KEY RESEARCH ACCOMPLISHMENTS

- ***Blockage of ras by dominant negative ras (N17) partially decreases MAPK activation in the MCF7/HRG clones suggesting the coexistence of other pathways induced by HRG, which synergistically activate MAPK. On the other hand it completely blocks MAPK activation when MCF7/V cells are treated with HRG [Figure 1].***

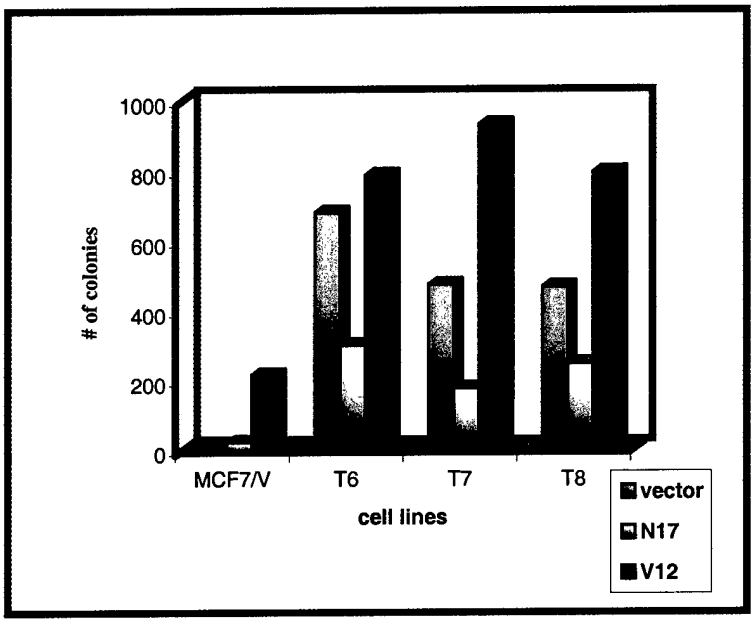
Figure 1: Detection of p21-ras, MAPK and phosphorylated MAPK by Western Blot.



MCF7/V and MCF7/HRG (T7 and T8) cells infected with vector alone (V), N17 or V12 were plated at 500Kcells/well in 6-well plates and the following day serum starved for 24 hrs. MCF7.V/V, MCF7.V/N17 and MCF7.V/V12 cells were also treated with 60 ng/ml of HRG for 15 minuets. Cell lysates (50μg/lane) were subjected to SDS-PAGE. Densitometric analysis of p42/44 MAPK phosphorylation levels.)

- ***N17ras reduced the ability of the HRG transfected clones to form colonies in absence of estrogen in Anchorage-independent growth assay by about 50%. Expression of constitutively active ras (V12) enhances colony formation of MCF7/V cells in absence of estrogen, as expected, however, increase of colony formation is much lower than in the cells transfected with HRG [Figure 2]***

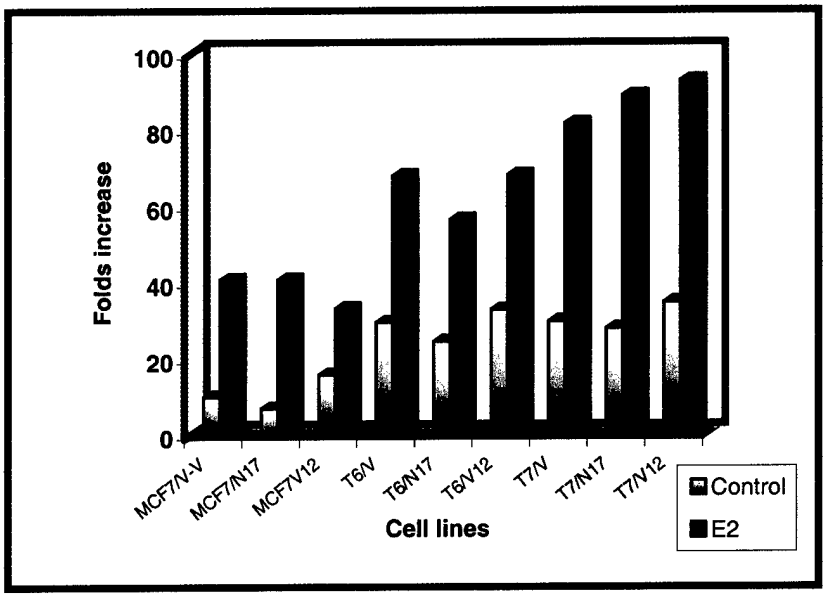
Figure 2: Effect of N17 and V12 ras constructs on Anchorage-independent growth



The ability of the cells to form colonies in the absence of E2 was assessed in soft agar assay. The cells were depleted from estrogen (E2) for 4 days prior to plating at 10000cells/well in soft agar. The cells were allowed to grow colonies for 14 days and then the colonies were stained sing 1mg/ml solution of *p*-Iodonitrotetrazolium violet.

- ***Expression of N17ras does not affect the anchorage-dependent growth of the MCF7/V or HRG transfected cell lines (T6 and T7). V12ras, like wise, does not affect the anchorage-dependent growth of the MCF7/HRG cell lines, but it slightly increases the growth of MCF7/V cells in the absence of E2 (1.5 folds increase induced by V12ras vs. more than 3 folds increase induced by HRG) [Figure 3].***

Figure 3. Effect of N17 and V12 ras constructs on Anchorage-dependent growth of MCF7 and MCF7/HRG (T6 and T7) cells.



All cells were starved from estrogen in charcoal-treated serum (CCS) containing media for 4 days prior to plating at 10000 cells/well. Cells were grown for 5 days in absence or presence of Estrogen (E2). Cell numbers was determined by counting using a coulter counter.

REPORTABLE OUTCOMES

Heregulin (HRG), a growth factor over expressed in about 30% of breast cancer tumors, has been associated with estrogen independence and metastasis of breast cancer cells. A recent study in our lab has shown that HRG activates the mitogen activated protein kinase (MAPK) signaling cascade when stably transfected into estrogen-dependent MCF7 cells. In addition, we showed that MAPK inhibitors specifically block HRG induced MAPK activity in breast cancer cells. We then hypothesized that HRG-induced activation of MAPK and progression of breast cancer cells to estrogen-independence may involve activation of the *ras*-signaling pathway. As a model, we infected MCF7 cells previously transfected with HRG (MCF7/HRG) with a retroviral-vector containing a dominant-negative mutant of *ras*, N17. Expression of the N17 mutant in MCF7/HRG cells was determined by western blot analysis. As expected, blockage of ras inhibited the activation of MAPK induced by HRG. Strikingly, it also inhibited colony formation of MCF7/HRG cells in anchorage-independent growth assay in the absence of estrogen and restored the estrogen dependence and antiestrogen-sensitivity of MCF-/HRG cells, but it did not alter the growth of the cells in an anchorage-dependent growth assay. To investigate whether *ras* alone is sufficient to confer estrogen independence, we transduced MCF7 cells with a retroviral-vector containing an activated *ras* (V12) sequence. This mutant, however, partially induced estrogen-independence. These results indicate that *ras* is a necessary mediator for the mechanism of HRG action, but it is not sufficient. Our results demonstrate that activation of *ras* and its cascade are potential targets to halt breast cancer progression and to revert estrogen-independence and antiestrogen resistance.

CONCLUSIONS

The inhibition of the Ras-Raf-MAPK pathway significantly reduces the aggressive phenotype of the HRG transfected cell lines in anchorage-independent growth assays. Therefore, our results suggest an important role for this pathway in the HRG induction of estrogen-independence in MCF7 breast cancer cell lines and demonstrate its components as potential targets for halting progression of breast cancer.

REFERENCES

None

APPENDICES

None